

SUPPLEMENTARY MATERIAL

Synthesis of side-chain modified peptides using iterative solid phase 'click' methodology

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|---|------|
| 1. General Experimental Information and synthetic procedures | pS2 |
| 2. Copies of NMR spectra for 1-7 | pS5 |
| 3. Anion titration data for 4•Zn₂ ; 6•Zn₂ and 7•Zn₂ | pS19 |

General

^1H NMR spectra were recorded at 300 K on a Bruker Avance III 500 at a frequency of 500.13 MHz, a Bruker Avance DPX 400 at a frequency of 400.13 MHz, a Bruker Avance DPX 300 at a frequency of 300.13 MHz or a Bruker Avance DPX 200 at a frequency of 200.13 MHz and are reported as parts per million (ppm) with the residual protons in deuterated solvents as internal references. ^1H NMR signals are reported as chemical shift values δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets and m = multiplet), coupling constant (J Hz) and relative integral. ^{13}C NMR were recorded using a Bruker Avance III 500 at a frequency of 125.76 MHz, a Bruker Avance DPX 400 at a frequency of 100.61 MHz or a Bruker Avance DPX 300 at a frequency of 75.47 MHz and are reported as parts per million (ppm) with CDCl_3 (δ_{C} 77.16 ppm), CD_3OD (δ_{C} 49.00 ppm) or $\text{DMSO}-d_6$ (δ_{C} 40.45 ppm) as an internal reference standard.

Melting points were measured using a Stanford Research Systems Optimelt melting point apparatus and are uncorrected. Optical rotations were performed at 20 °C using the indicated spectroscopic grade solvent on a Perkin Elmer model 341 polarimeter or a PO LAR 2001 polarimeter at 589 nm. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer using attenuated total reflection (ATR) of a thin film.

Low resolution mass spectra were recorded on a Thermo Finnigan LCQ Deca Ion Trap mass spectrometer and high resolution mass spectra were recorded on a 4.7 T Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR). Ionization of all samples was carried out using electrospray ionisation (ESI) or atmospheric pressure chemical ionization (APCI).

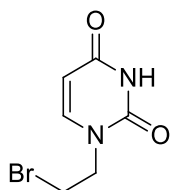
Liquid chromatography mass spectrometry (LCMS) data was obtained on a Shimadzu Separation Products: Spectra System based on a P400 Pump, a UV6000LP photodiode array detector, a Phenomenex Jupiter column (5 μm , 2.1 \times 150mm) and a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI). Flow rate was maintained at 0.2 mL min^{-1} with mobile phases of 0.1% formic acid in Milli-Q water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Analytical reverse phase high performance liquid chromatography (analytical

RP-HPLC) was performed on a Waters 2695 separations module with an Alliance series column heater, a Waters SunFire™ C18 column (5 μm, 2.1 × 150 mm) and a Waters 2996 photodiode array detector. The experiments were carried out at 30 °C with a flow rate maintained at 0.2 mL/min (mobile phases solvent A: 0.1% formic acid in Milli-Q water and solvent B: 0.1% formic acid in acetonitrile). Preparative RP-HPLC was performed on a Waters 600 controller with a Waters 600 pump, a 2998 photodiode array detector and Waters Empower 2 software. Separation was achieved on a XBridge™ Prep Shield C18 OBD™ (5 μm, 19 × 150 mm) column at a flow rate of 7.0 mL min⁻¹, using mobile phases of 0.05% ammonia in Milli-Q water (solvent A) and 0.05% ammonia in acetonitrile (solvent B). The collected fractions from preparative HPLC were lyophilized using a Labconco FreeZone 6 liter console freeze dry system after removal of acetonitrile.

Fluorescence spectra were recorded using a Varian Cary Eclipse Fluorescence Spectrophotometer. UV-Vis data was recorded using a Varian Cary 4000 UV-Vis Spectrophotometer. Temperature control was provided by a Varian Cary PCB 150 Water Peltier System and pH values were determined using an Activon Model 209 pH/mV meter.

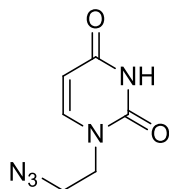
Synthesis

1-(2-Bromoethyl)uracil (**15**)



Uracil (1.00 g, 8.9 mmol) and ClSiMe₃ (0.54 mL, 4.5 mmol) in hexamethyldisilazane (5.7 mL, 27.0 mmol) were allowed to reflux under argon for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in DMF (5 mL) followed by addition of 1,2-dibromoethane (2.3 mL). The resulting mixture was heated at 80 °C for 24 h, cooled and treated with water (80 mL). After filtration, the aqueous filtrate was then extracted with CH₂Cl₂ (3 x 200 mL). The combined organic extracts were dried over MgSO₄ and concentrated to afford the product **15** (0.60 g, 31%). ¹H NMR (300 MHz, CDCl₃): δ 8.81 (s, 1H), 7.29 (d, *J* = 7.8 Hz, 1H), 5.75 (d, *J* = 7.8 Hz, 1H), 4.16 (t, *J* = 5.8 Hz, 2H), 3.70 (t, *J* = 5.8 Hz, 2H); HRMS (ESI) calcd. for C₆H₇BrN₂O₂Na [M + Na]⁺ 240.9583, found 240.9584.

1-(2-Azidoethyl)uracil (**14**)



A mixture of **15** (0.60 g, 2.7 mmol), sodium azide (0.51 mg, 8.2 mmol) and tetrabutylammonium chloride (0.76 mg, 2.7 mmol) in acetone (3 mL) and water (3 mL) was allowed to stir at rt for 40 h.¹⁵⁰ Extracted with ethyl acetate (5 x 20 mL), the combined organic layers were dried and evaporated to give a gum which was then purified by flash column chromatography (silica gel; 100% ethyl acetate). Compound **14** was obtained as a colourless solid (0.26 g, 52%). IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3263, 3014, 2157, 1660, 1478, 1416, 1206, 837; ^1H NMR (200 MHz, CDCl_3): δ 7.34 (d, $J = 8.0$ Hz, 1H), 5.61 (d, $J = 8.0$ Hz, 1H), 3.86 (t, $J = 5.0$ Hz, 2H), 3.60 (t, $J = 5.0$ Hz, 2H).



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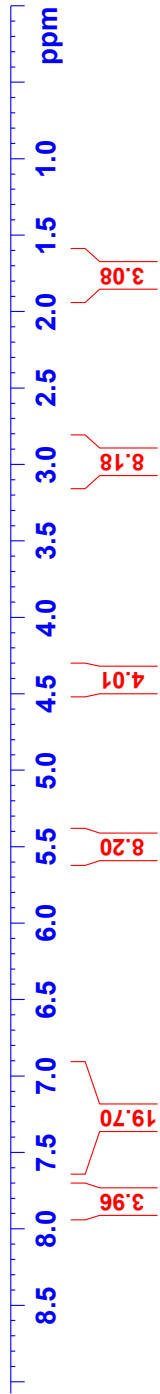
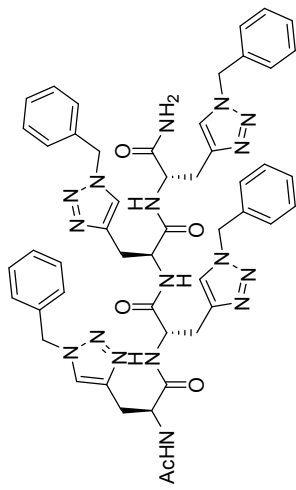
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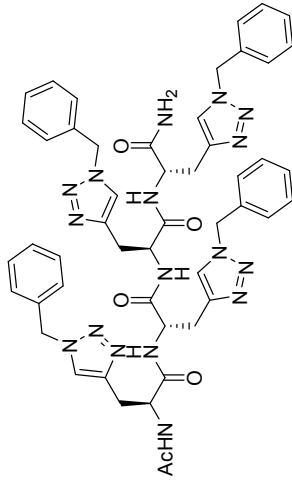
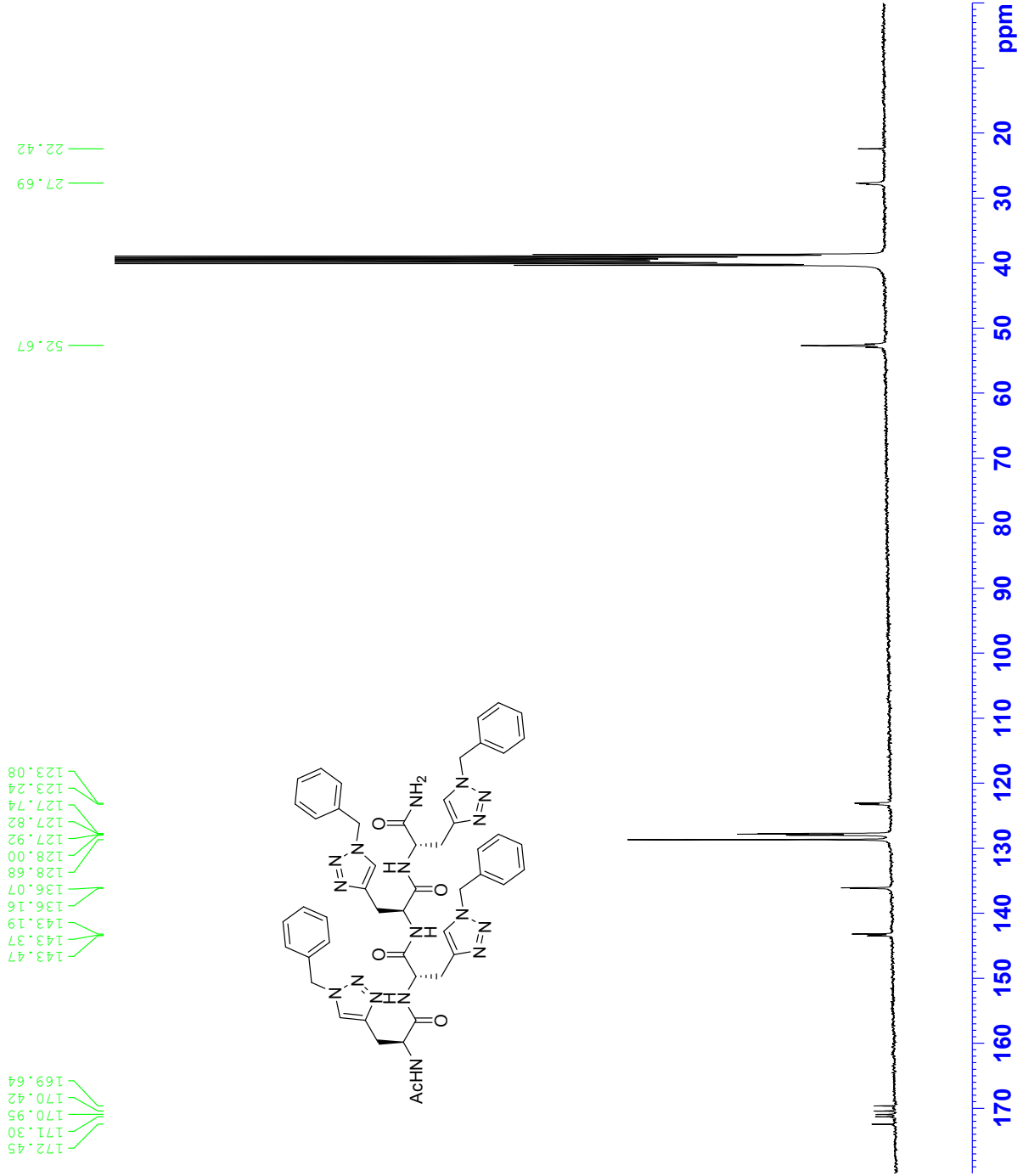
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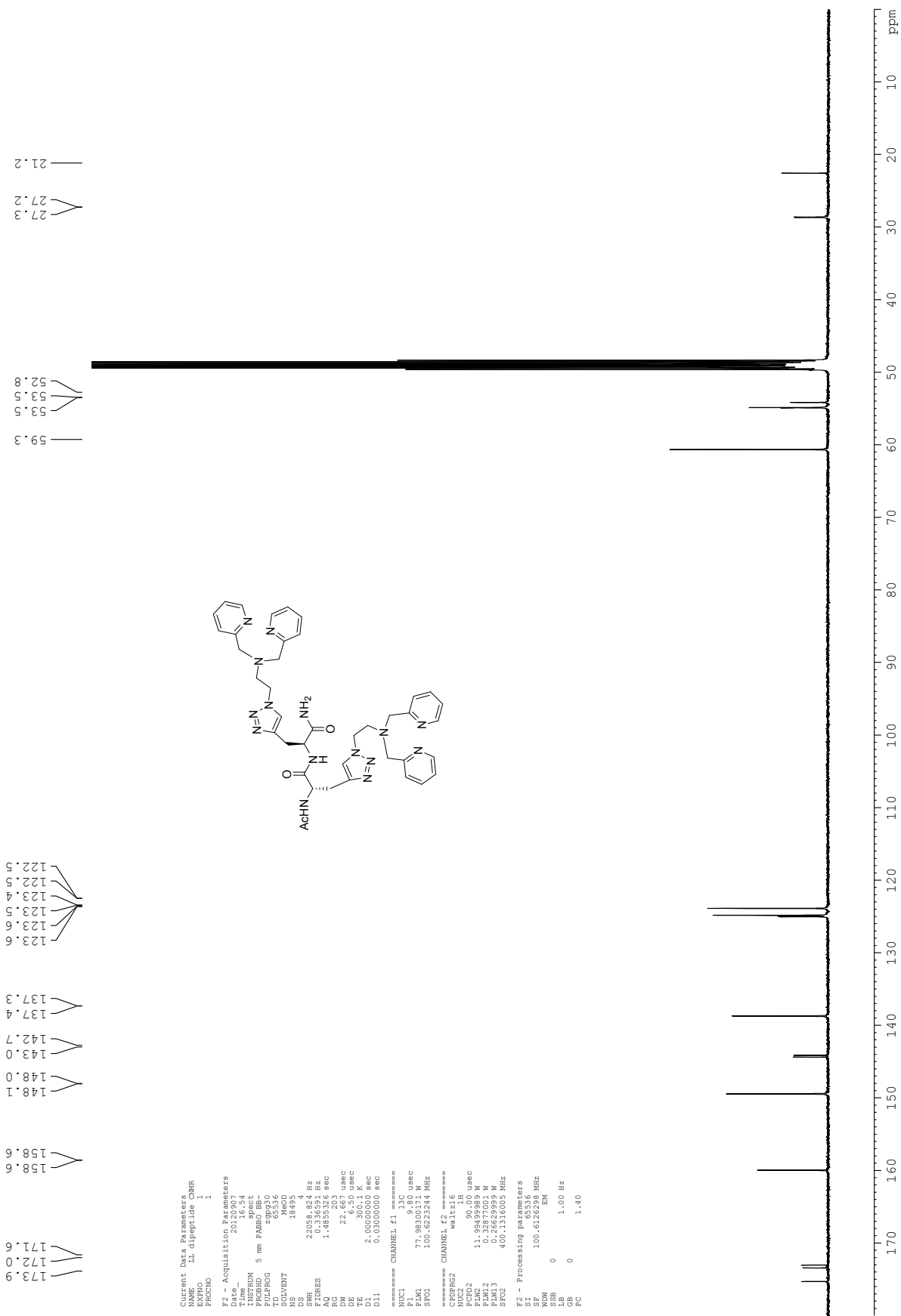
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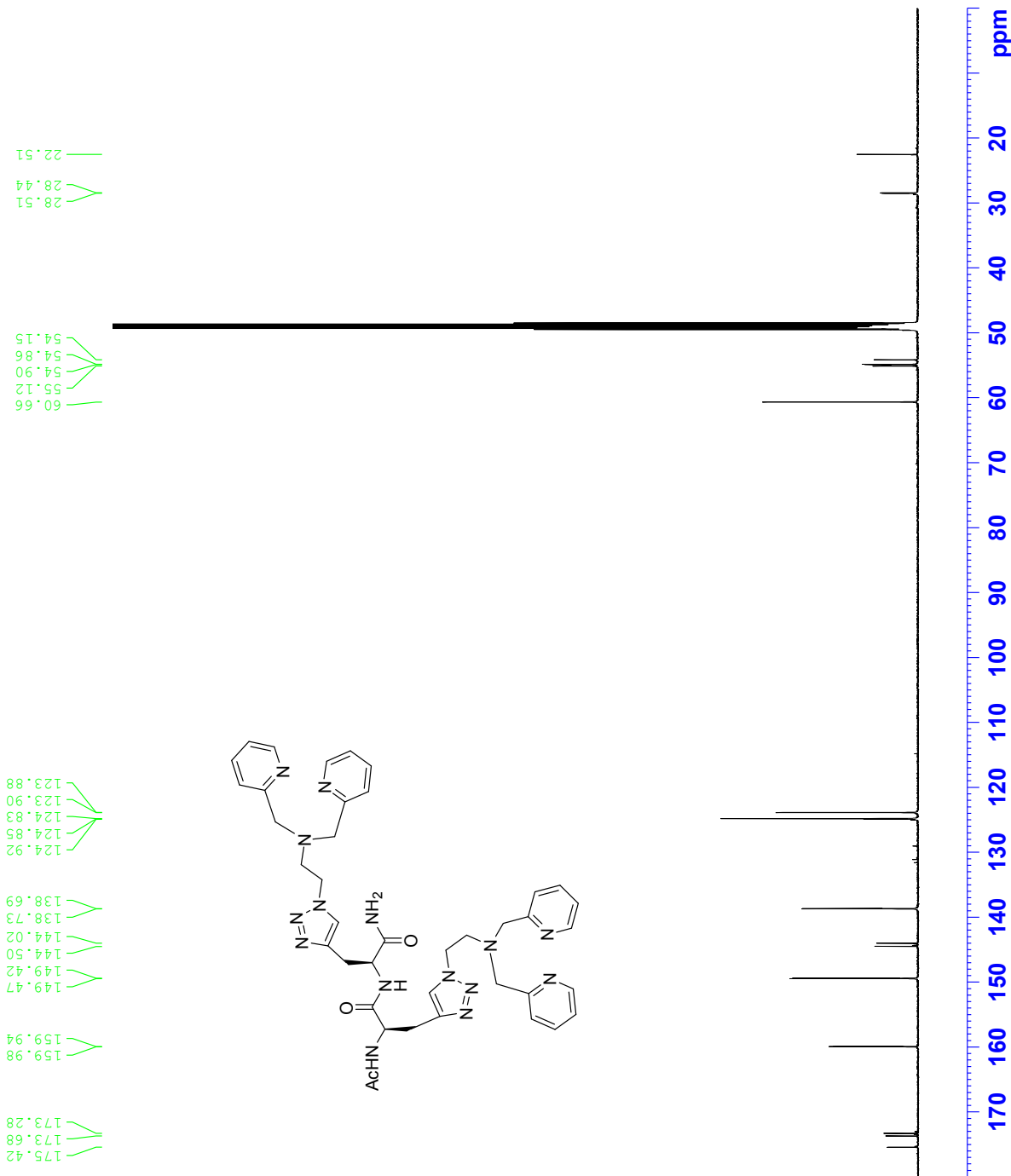
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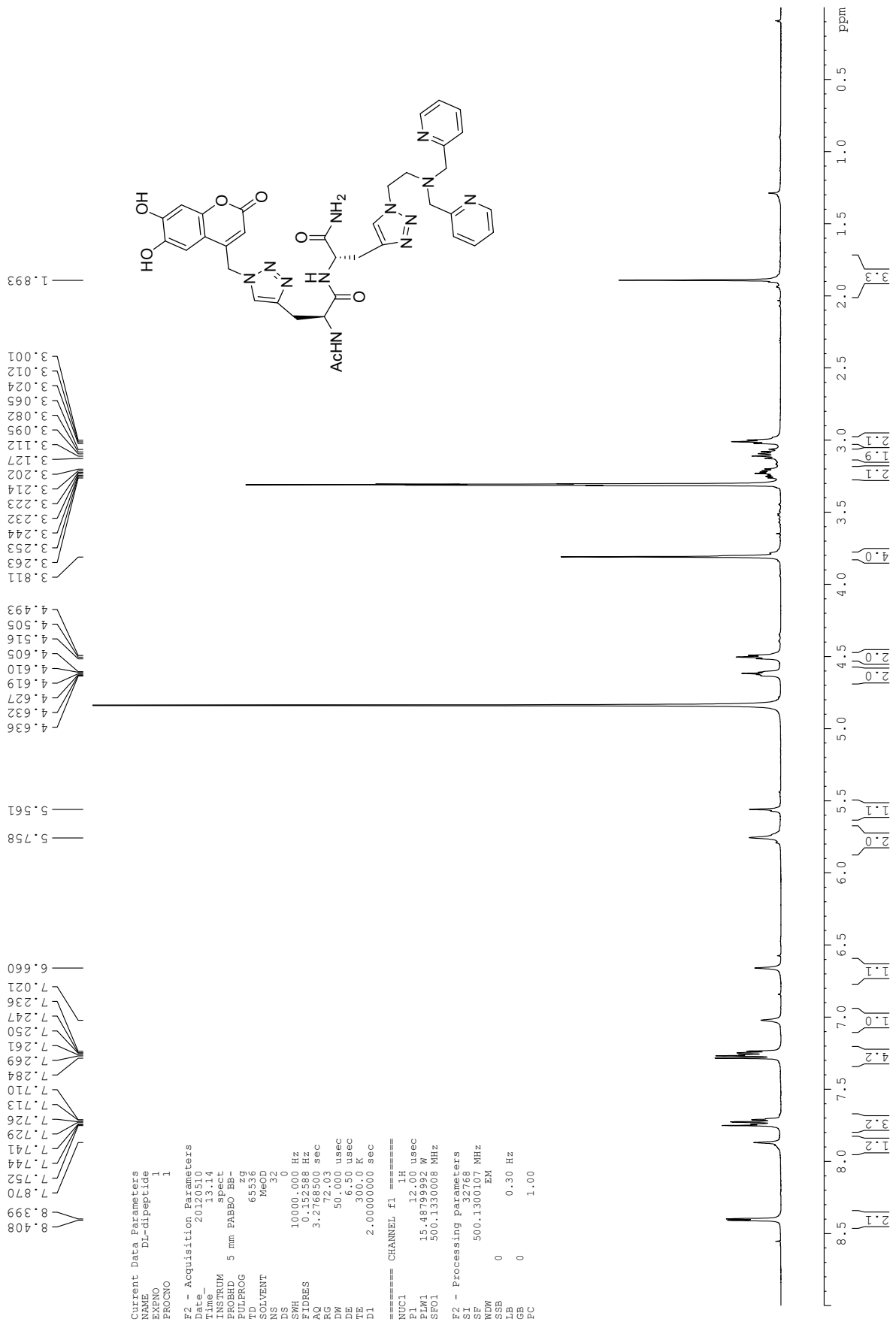
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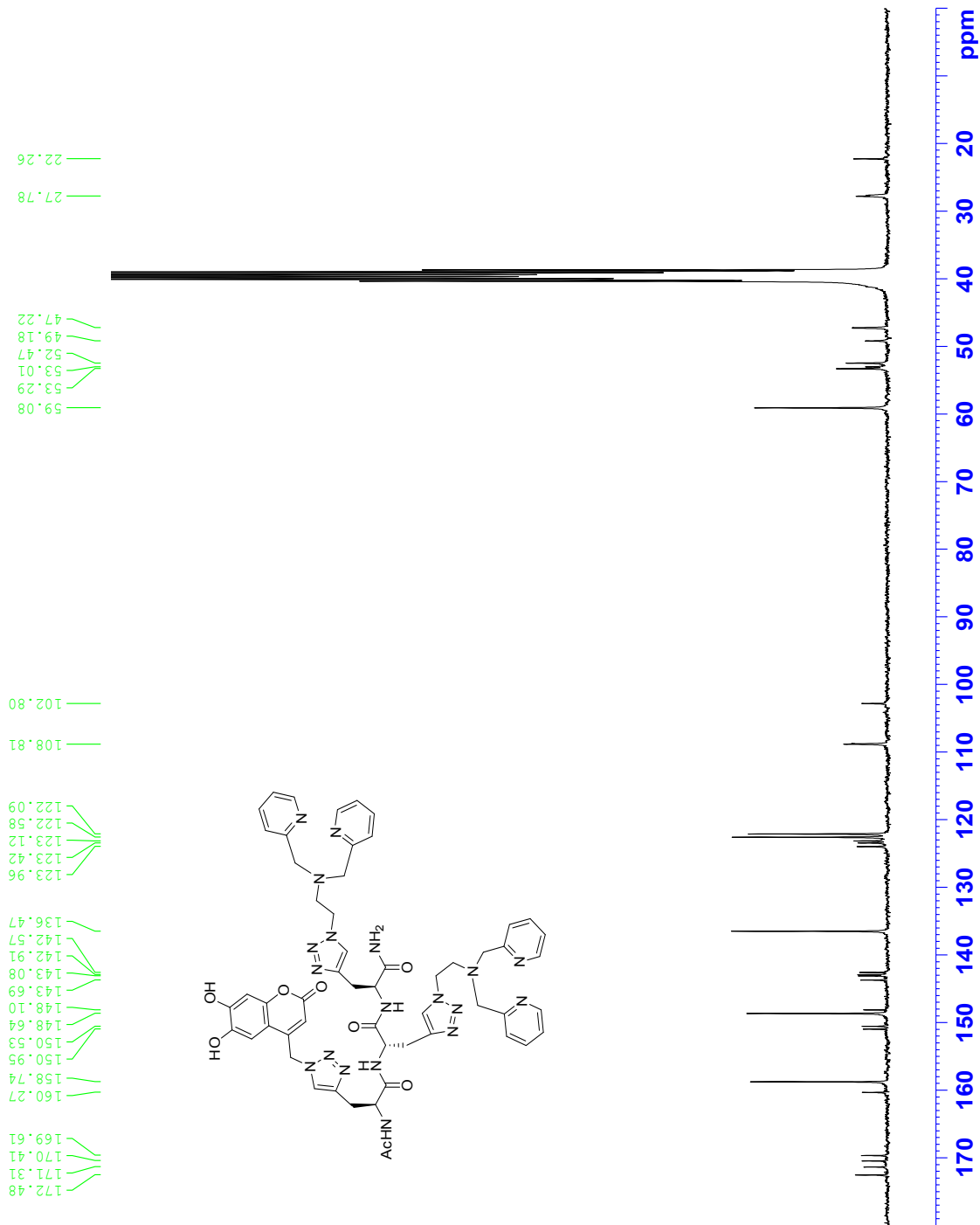
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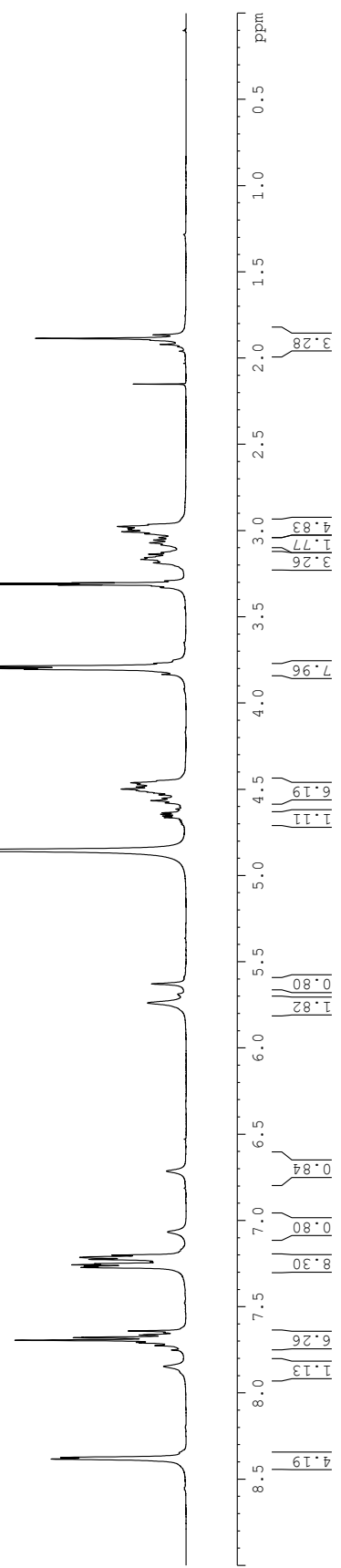
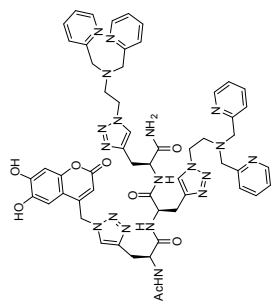
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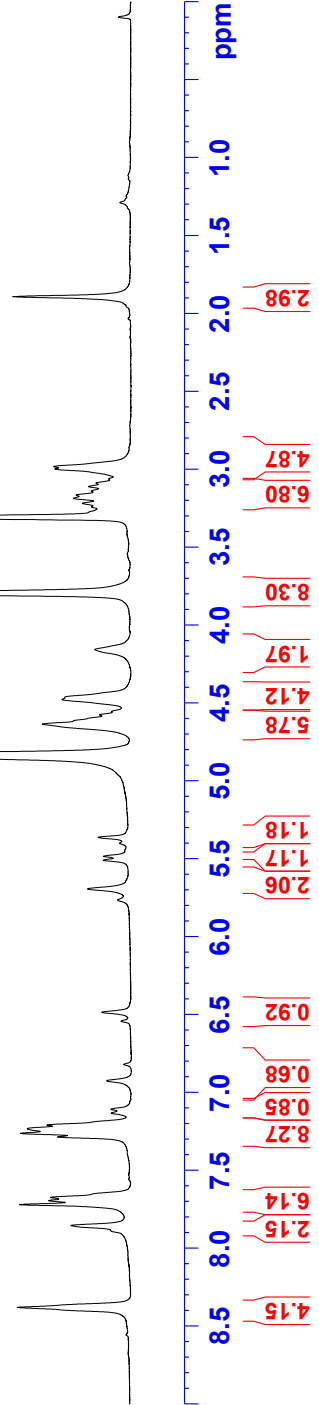
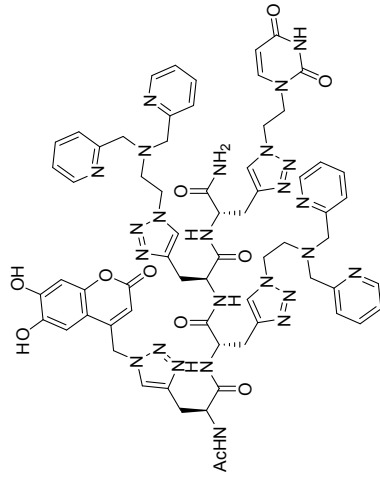


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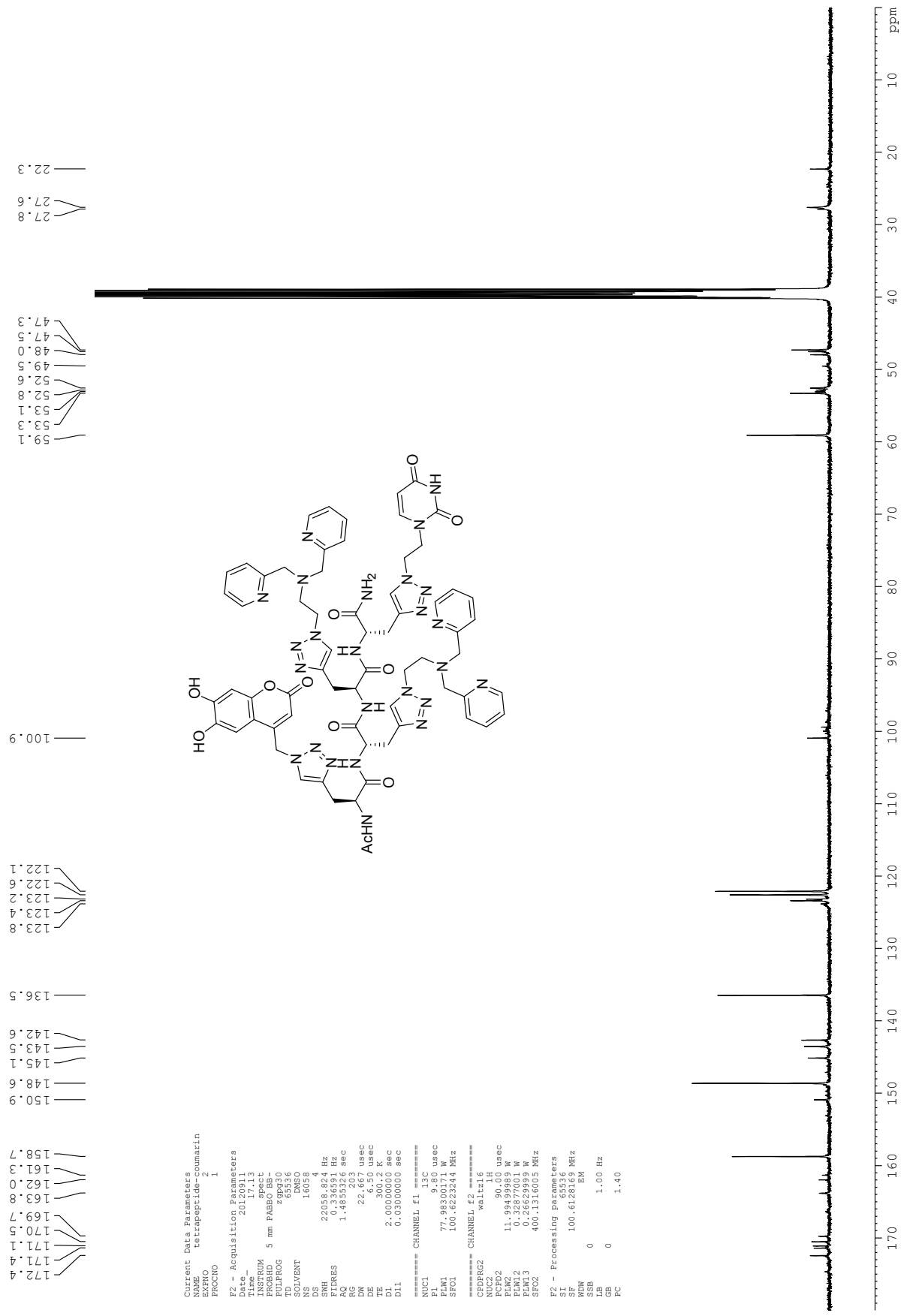
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Fluorescence titration data

Compound $4 \cdot \text{Zn}_2$

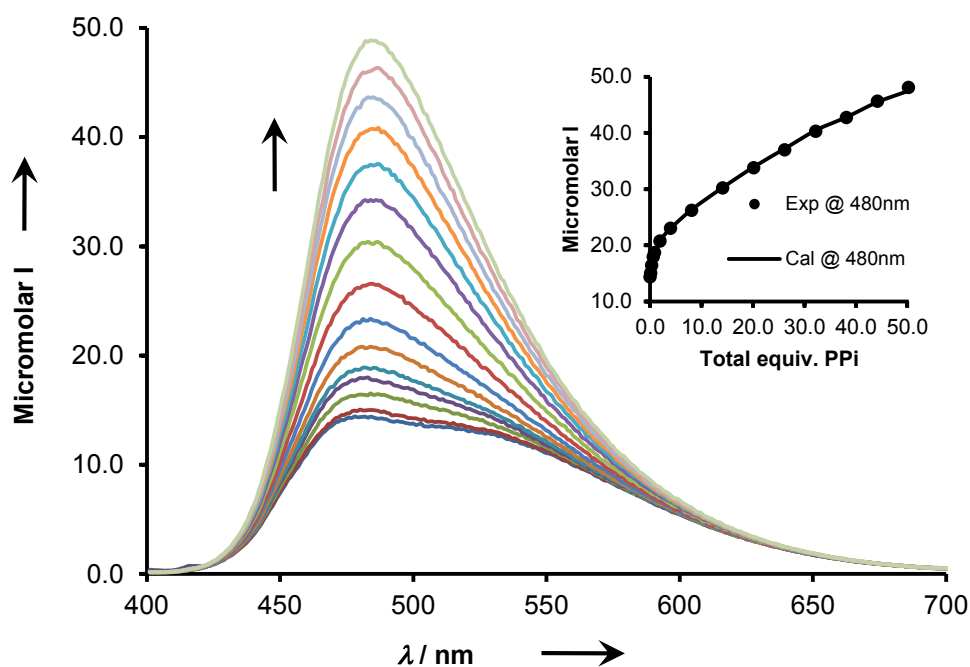


Figure S1 Changes in fluorescence intensity of $4 \cdot \text{Zn}_2$ ($5 \mu\text{M}$) upon addition of addition of PPI (sodium salt), $[\text{PPI}] = 0.5, 1.5, 3.0, 4.5, 9.6, 20.0, 40.6, 70.7, 100.8, 130.9, 161.0, 191.1, 221.2, 251.3 \mu\text{M}$ in aqueous solutions of HEPES buffer ($5 \text{ mM}, 145 \text{ mM NaCl}, \text{pH } 7.4$) at 25°C , $\lambda_{\text{ex}} = 390 \text{ nm}$, slit 5/5. Inset: 1:1 fitting curve at 480 nm.

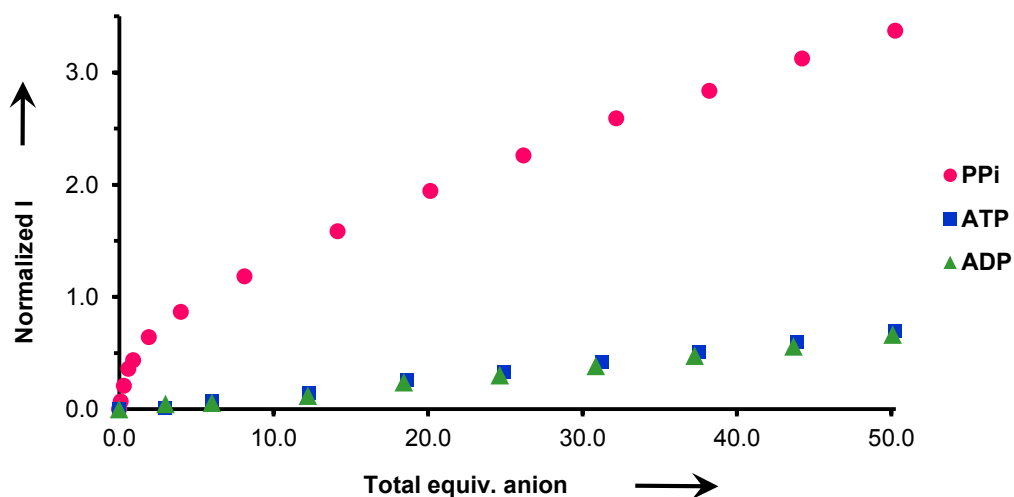


Figure S2 Changes in fluorescence intensity for of $4 \cdot \text{Zn}_2$ ($5 \mu\text{M}$) at 480 nm upon addition of up to 50 equiv. of anions (sodium salts) in aqueous solutions of HEPES buffer ($5 \text{ mM}, 145 \text{ mM NaCl}, \text{pH } 7.4$) at 25°C , $\lambda_{\text{ex}} = 390 \text{ nm}$, slit 5/5. I = normalized fluorescence intensity.

Compound $6 \cdot \text{Zn}_2$

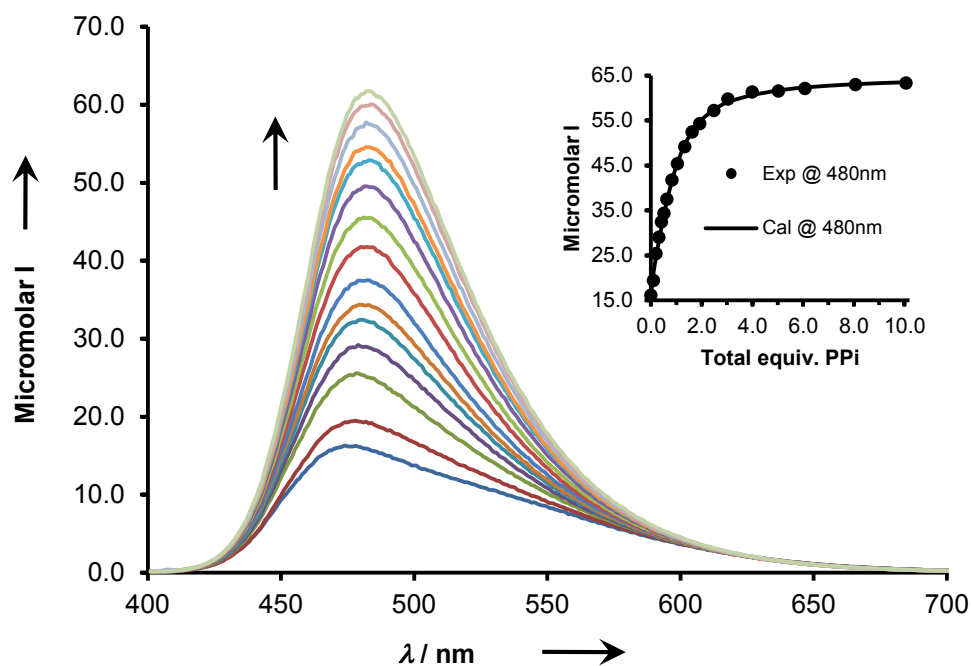


Figure S3 Changes in fluorescence intensity of $6 \cdot \text{Zn}_2$ (5 μM) upon addition of addition of PPI (sodium salt), [PPI] = 0.5, 1.0, 2.1, 2.6, 3.1, 4.2, 5.2, 6.7, 8.2, 9.6, 12.4, 15.2, 20.0, 25.2, 30.4, 40.3, 50.3 μM in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 $^\circ\text{C}$, $\lambda_{\text{ex}} = 390 \text{ nm}$, slit 5/5. Inset: 1:1 fitting curve at 480 nm.

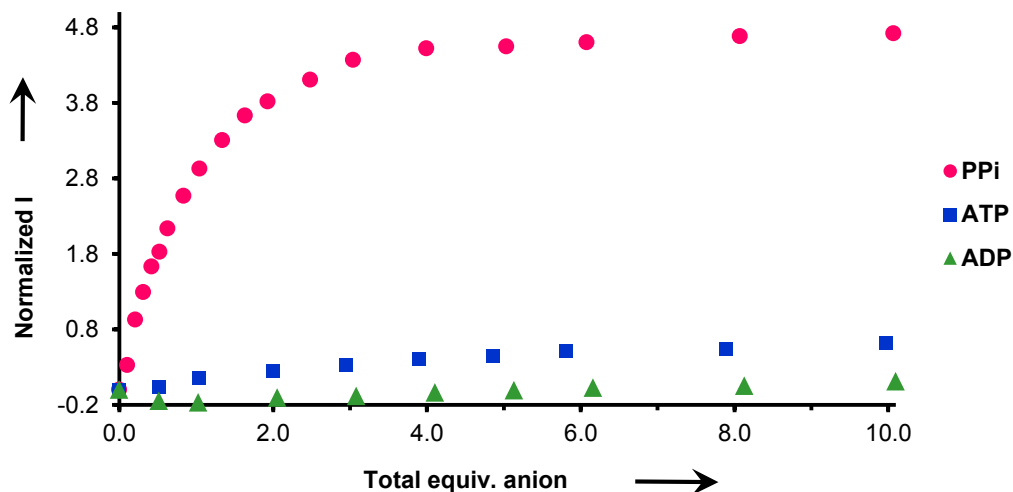


Figure S4 Changes in fluorescence intensity of $6 \cdot \text{Zn}_2$ (5 μM) at 480 nm upon addition of up to 10 equiv. of anions (sodium salts) in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 $^\circ\text{C}$, $\lambda_{\text{ex}} = 390 \text{ nm}$, slit 5/5. I = normalized fluorescence intensity.

Compound $7 \cdot \text{Zn}_2$

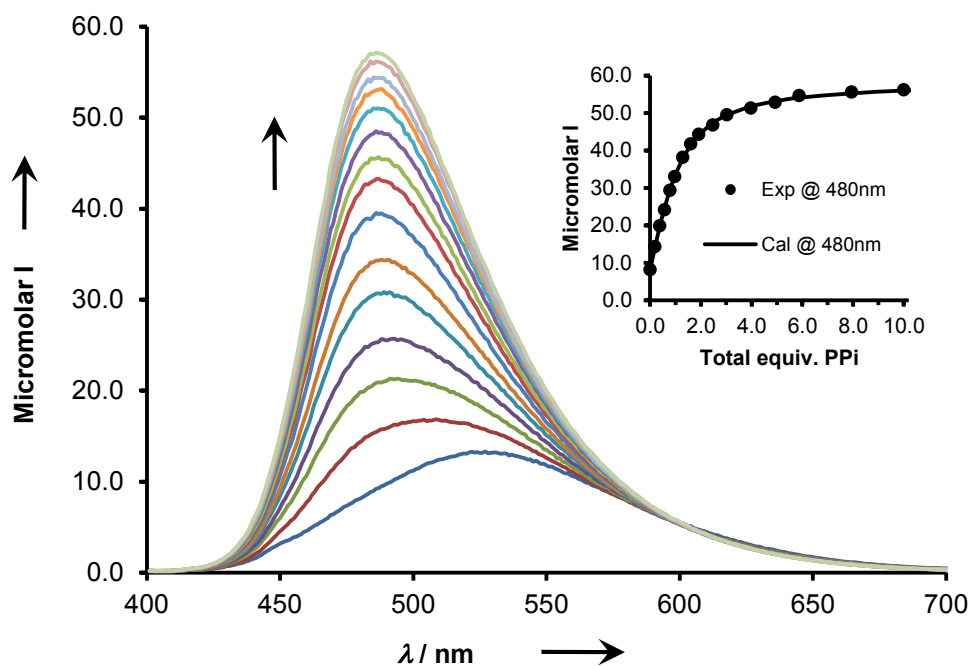


Figure S5 Changes in fluorescence intensity of $7 \cdot \text{Zn}_2$ (5 μM) upon addition of addition of PPI (sodium salt), [PPI] = 1.0, 1.9, 2.9, 3.9, 4.8, 6.4, 8.0, 9.6, 12.4, 15.1, 19.9, 24.6, 29.4, 39.7, 50.0 μM in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 $^\circ\text{C}$, $\lambda_{\text{ex}} = 390 \text{ nm}$, slit 5/5. Inset: 1:1 fitting curve at 480 nm.

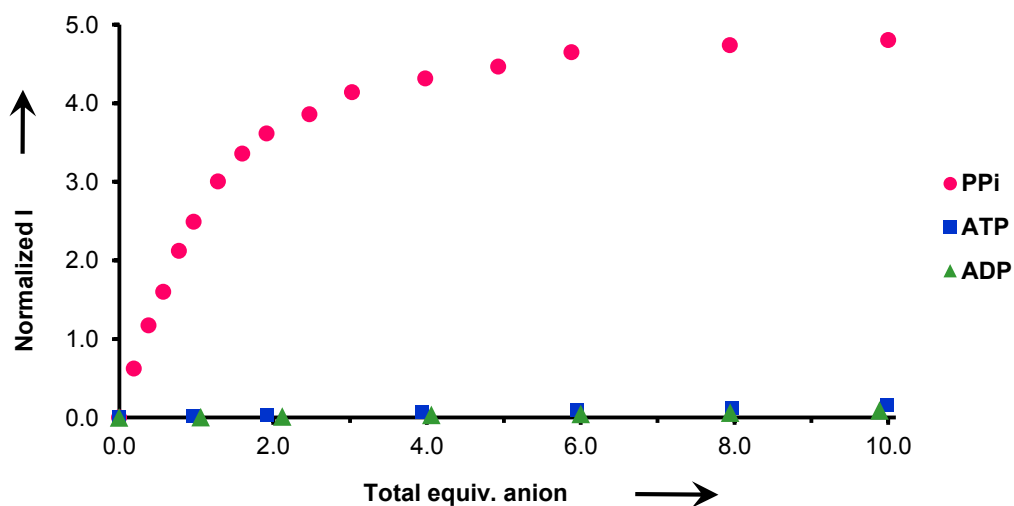


Figure S6 Changes in fluorescence intensity of $7 \cdot \text{Zn}_2$ (5 μM) at 480 nm upon addition of up to 10 equiv. of anions (sodium salts) in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 $^\circ\text{C}$, $\lambda_{\text{ex}} = 390 \text{ nm}$, slit 5/5. I = normalized fluorescence intensity.